PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Thomas L. Benjamin et al.	Art Unit:	1632
Serial No.:	09/988,117	Examiner:	Q. Janice Li
Filed:	November 16, 2001	Customer No.:	21559
Title:	DIAGNOSING AND TREATING CANCER CELLS USING SAL2		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. THOMAS BENJAMIN, Ph.D.

Under 37 C.F.R. § 1.132 and regarding the rejection of claims 1-10, I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application.

2. I have read the Office Action mailed on January 29, 2003 in connection with the above-referenced patent application

3. We have examined the biological role and molecular function of $p150^{Sal2}$ in cancer progression using techniques known in the art at the time the application was filed and have demonstrated an association between loss of $p150^{Sal2}$ expression and cancer. In this regard, we show that while $p150^{Sal2}$ is frequently absent in human

ovarian carcinomas (OVCA) cells (EXHIBIT 1B), it is highly expressed in normal ovarian surface epithelium (HOSE) cells (see EXHIBIT 1A). Analysis of 17 clinical ovarian tumor extracts further confirmed the loss of $p150^{Sal2}$ expression in the majority (13 out of 17) of tumors (EXHIBIT 1C). Based on our analysis of ovary tumor and normal tissue screened to date, loss of expression of $p150^{Sal2}$ expression is clearly associated with the incidence of cancer. 4. We have also shown that restoration of $p150^{Sal2}$ expression is effective in reducing replication and viability of tumor cells in a p53-independent manner. We show that SKOV-3, a human OVCA-derived cell line that is p53 null and that produces tumors in nude mice having a histology similar to that of human ovarian carcinomas, expresses only trace amounts of $p150^{Sal2}$ compared to HOSE cells (EXHIBIT 2E). Upon reintroduction of $p150^{Sal2}$ in SKOV-3 cells, DNA synthesis is dramatically reduced (presented as a percentage of BrdU negative cells among total of transfected cells in EXHIBIT 2A), the rate of apoptosis is significantly increased (EXHIBIT 2B), and colony formation is suppressed relative to SKOV-3 cells transfected with an empty vector (EXHIBIT 2C). Restoration of $p150^{Sal2}$ is also associated with an induction of p21 and Bax (EXHIBIT 3A and 3B), both of which are often down-regulated in cancer. Our results further demonstrate that $p150^{Sal2}$ binds to the p21 promoter and stimulates p21 transcription independently of p53 (EXHIBIT 3C, 3D, and 4A-4D). Conversely, a defect in $p150^{Sal2}$ expression (e.g., loss of heterozygosity at amino acid position 73)

results in a diminished ability to induce the p21 promoter (EXHIBIT 5). *In vivo,* the size of SKOV-3 tumors in which $p150^{Sal2}$ expression has been restored is significantly reduced relative to SKOV-3 tumors expressing the empty vector (EXHIBIT 2F and 2G) and is concomitant with an increase in apoptosis and a reduction in mitosis (EXHIBIT 2H and 2I). Conversely, the targeted reduction of $p150^{Sal2}$ expression by RNA interference in HOSE cells significantly increases DNA synthesis (EXHIBIT 2D). Our finding that demethylation of $p150^{Sal2}$ results in an increase in $p150^{Sal2}$ expression in some ovarian cancer cell lines further supports its role as a tumor suppressor given that the expression of tumor suppressors is often repressed by methylation in cancer (EXHIBIT 6). Taken together, the teachings disclosed herein are highly indicative that restoration of $p150^{Sal2}$ expression in tumor cells having a defect in Sal2 expression at the protein or RNA level, such as human ovarian tumor cells, would result in an increase in tumor cell apoptosis, a decrease in tumor cell proliferation, or both such that tumor growth would be prevented, reduced, or treated. Tumor cells that would particularly benefit from this treatment strategy include any cell that has a proliferative-associated alteration in the *Sal2* gene (e.g., S73C), reduced $p150^{Sal2}$ levels, or a $p150^{Sal2}$ protein of altered molecular weight. A person of ordinary skill in the field of the invention could have broadly practiced the invention as claimed by using the teachings in the present patent application and knowledge and techniques known in the field at the time of the

invention.

5. We also show that the induction in $p150^{Sal2}$ expression can markedly reduce the replication of HPV-16 DNA (EXHIBIT 7). C33A cells were transfected with a plasmid containing a viral origin (Ori), which is replicated when the HPV viral replication proteins E1 and E2 are expressed along with the Ori plasmid (second lane, upper band). When $p150^{Sal2}$ is expressed, replication is significantly suppressed (third and fourth lanes, upper band). The amount of suppression is indicated as a percentage based on the ratio of the upper band (replicated DNA) to the lower band (non-replicated DNA) for each lane. According to this finding, a reduction in DNA tumor virus replication and dissemination could be achieved by the administration of $p150^{Sal2}$ to cells infected with a DNA virus.

6. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

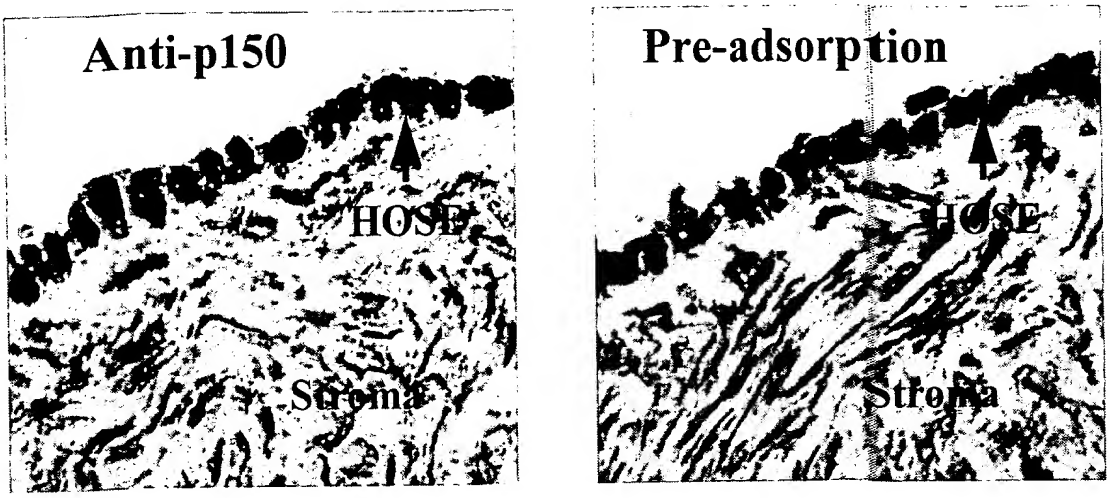
7/17/03
Date

Thomas Benjamin, Ph.D.

Exhibit 1

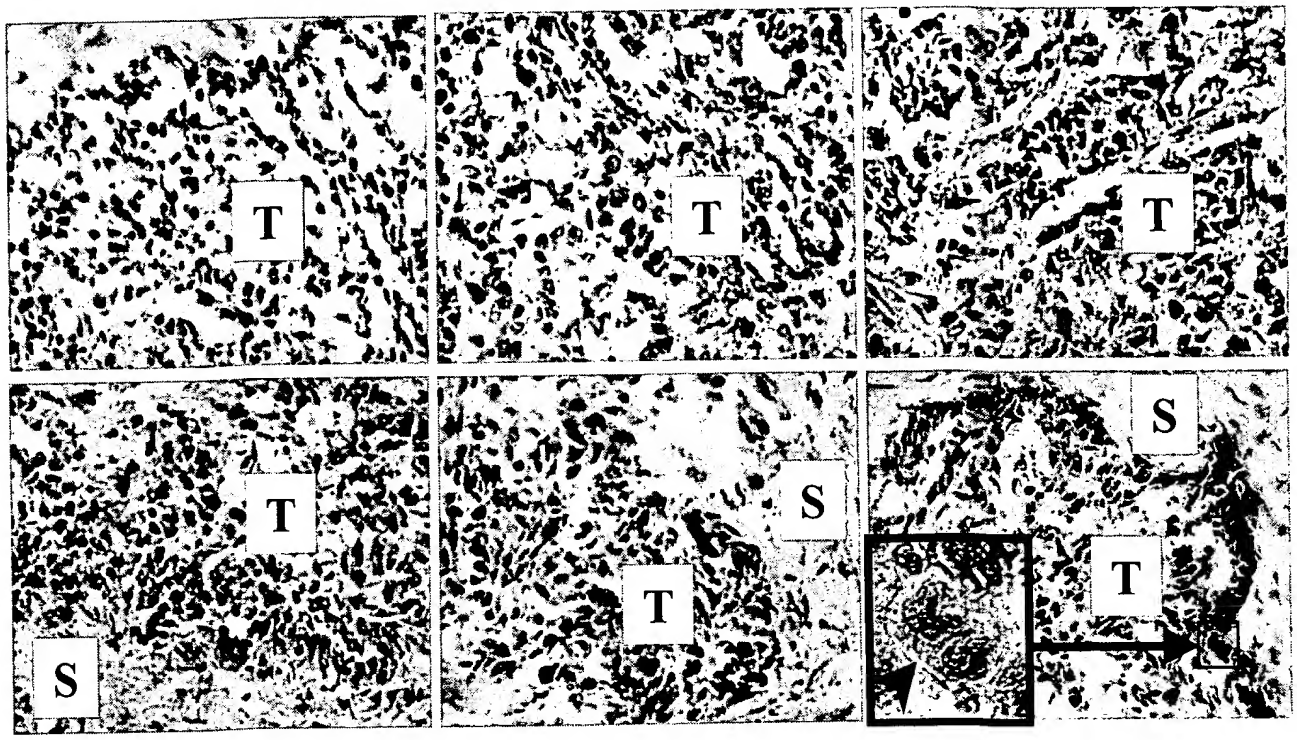
a

Normal Human Ovary



b

Human Ovarian Tumors



c

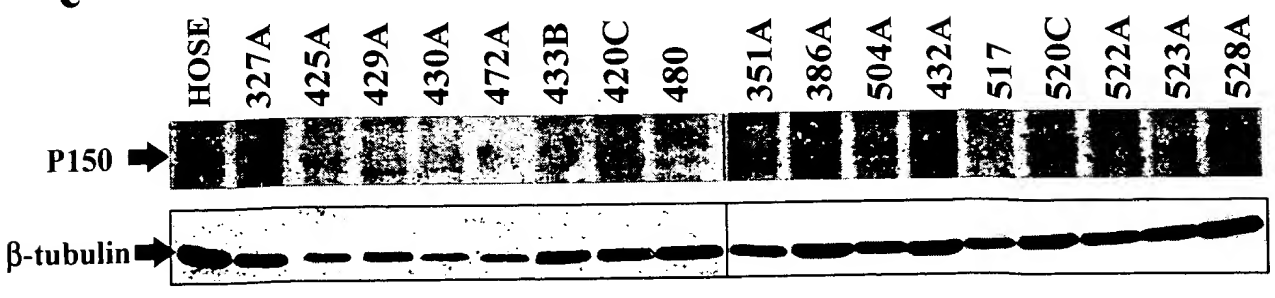


Exhibit 2

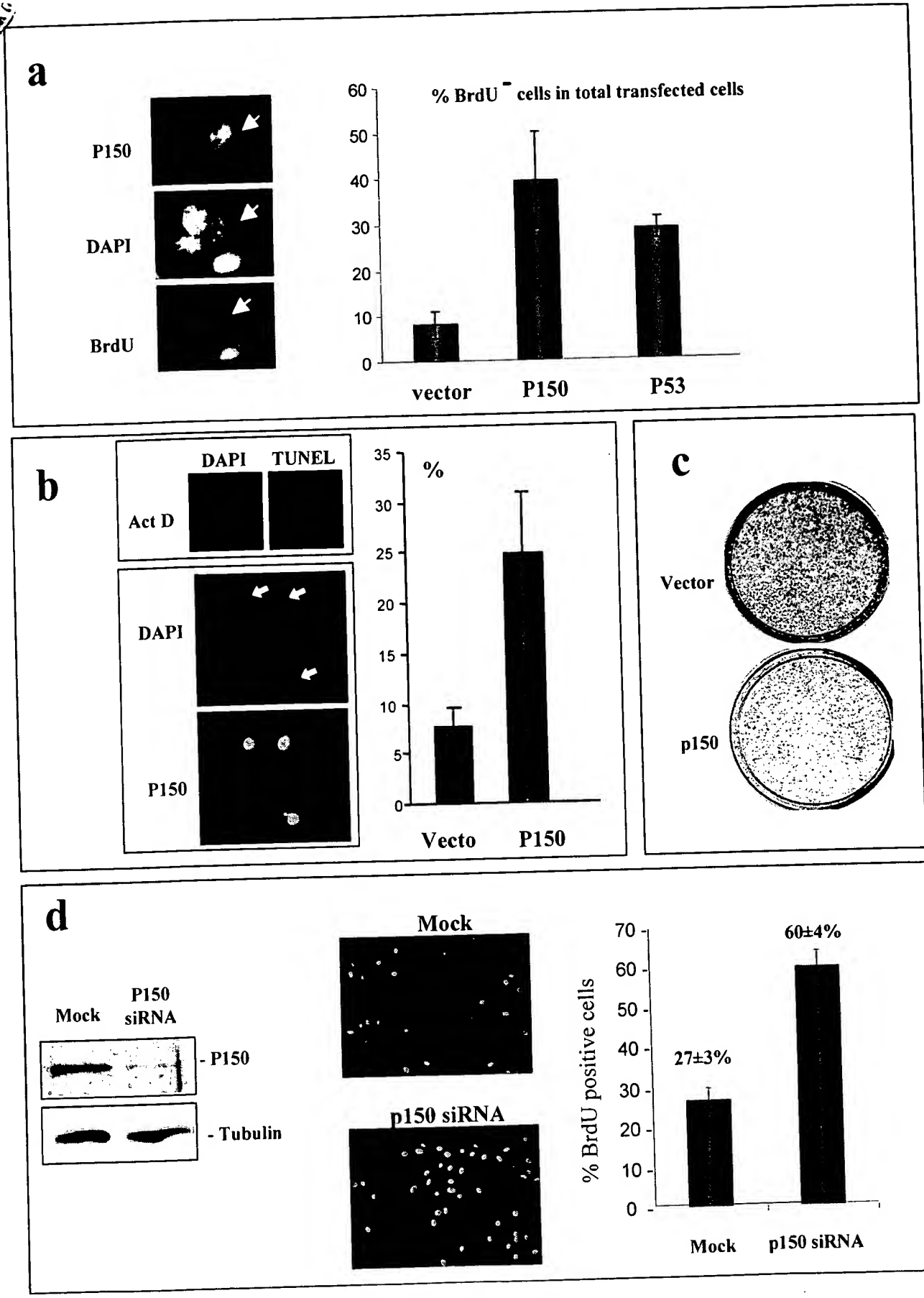


Exhibit 2

Continued

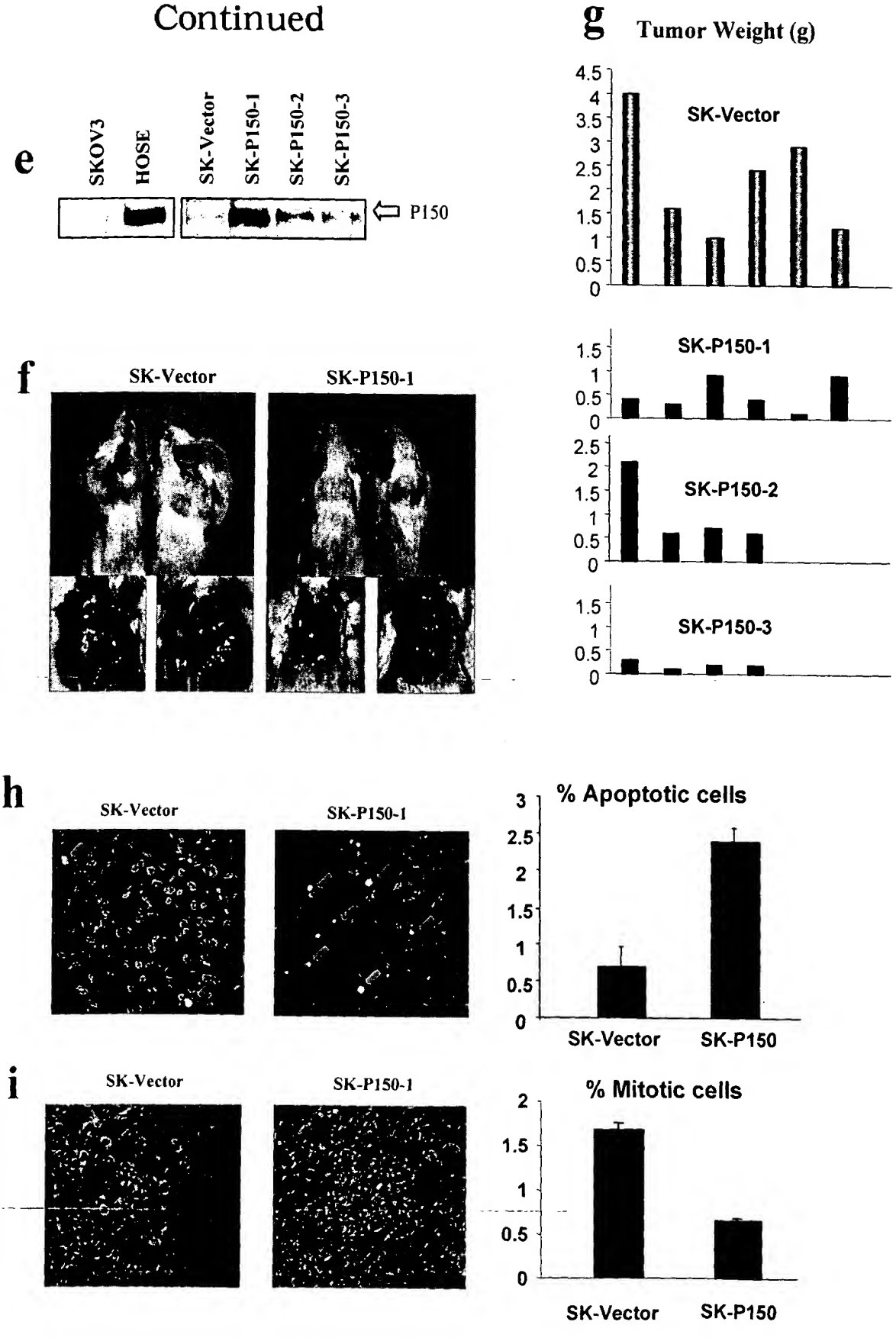


Exhibit 3

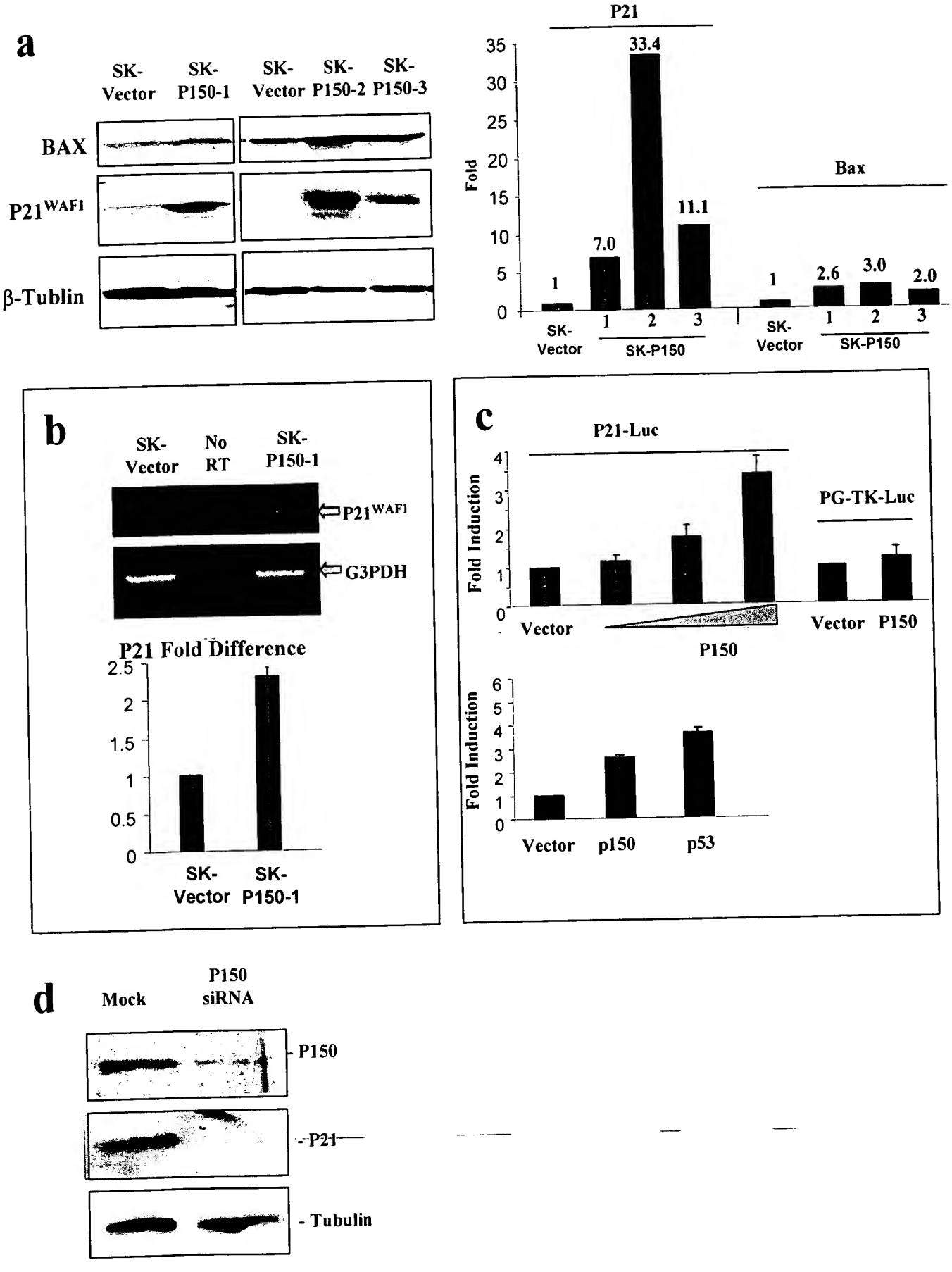
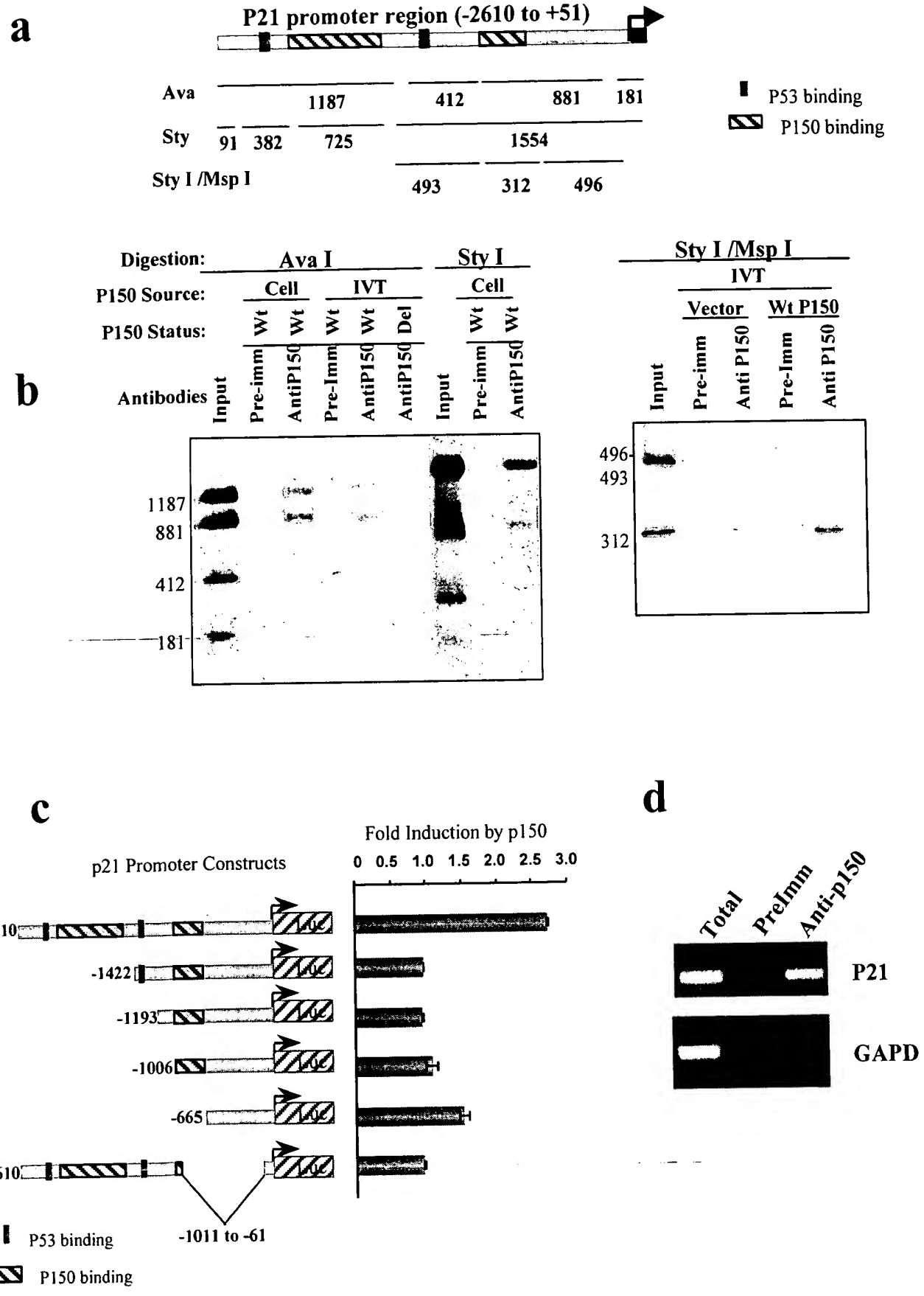
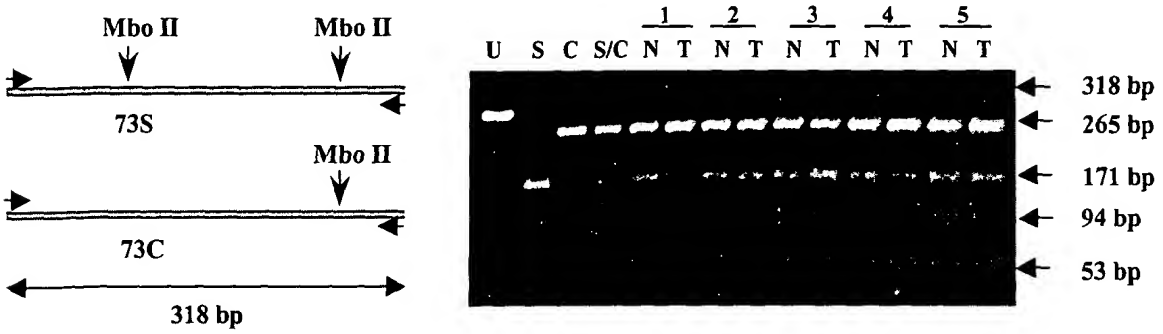

a
SK-Vector
SK-P150-1
SK-Vector
SK-P150-2
SK-P150-3
BAX
P21WAF1
β-Tublin
P21
Fold
33.4
11.1
7.0
1
SK-Vector
SK-P150
Bax
2.6
3.0
2.0
b
SK-Vector
No RT
SK-P150-1
P21WAF1
G3PDH
P21 Fold Difference
Fold Induction
c
P21-Luc
PG-TK-Luc
Vector
P150
p150
p53
d
Mock
P150 siRNA
P150
P21
Tubulin

Exhibit 4


a
P21 promoter region (-2610 to +51)
Ava
1187
412
881
181
Sty
91
382
725
1554
Sty I /Msp I
493
312
496
P53 binding
P150 binding
b
Digestion:
Ava I
Sty I
Sty I /Msp I
P150 Source:
Cell
IVT
Cell
IVT
P150 Status:
Wt
Wt
Wt
Wt
Del
Wt
Wt
Vector
Wt P150
Antibodies
Input
Pre-imm
AntiP150
Pre-Imm
AntiP150
AntiP150
Input
Pre-imm
AntiP150
Input
Pre-imm
Anti P150
Pre-Imm
Anti P150
1187
881
412
181
496
493
312
c
p21 Promoter Constructs
Fold Induction by p150
0 0.5 1.0 1.5 2.0 2.5 3.0
-2610
-1422
-1193
-1006
-665
-2610
-1011 to -61
P53 binding
P150 binding
d
Total
PreImm
Anti-p150
P21
GAPD

A p150 variant found in human ovarian tumor abolishes induction of p21 promoter

A: p150 LOH in the ovarian tumor of a 73 S/C patient



B: Effect of the 73 C variant on p21 promoter induction

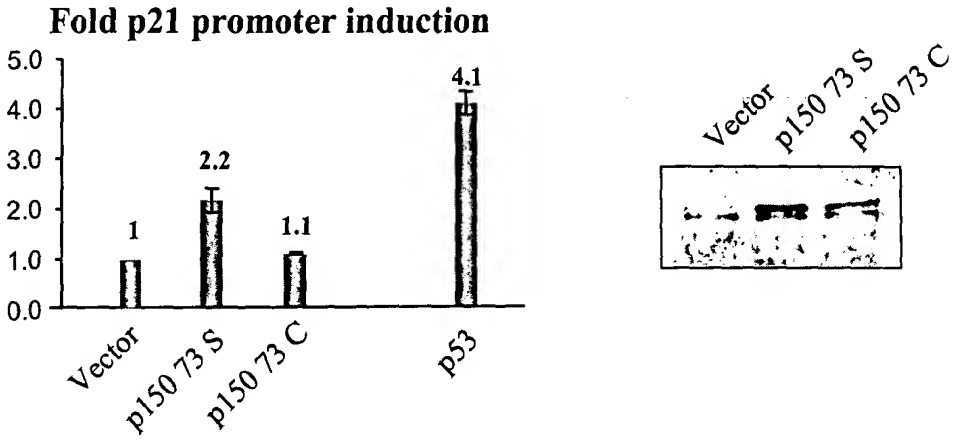
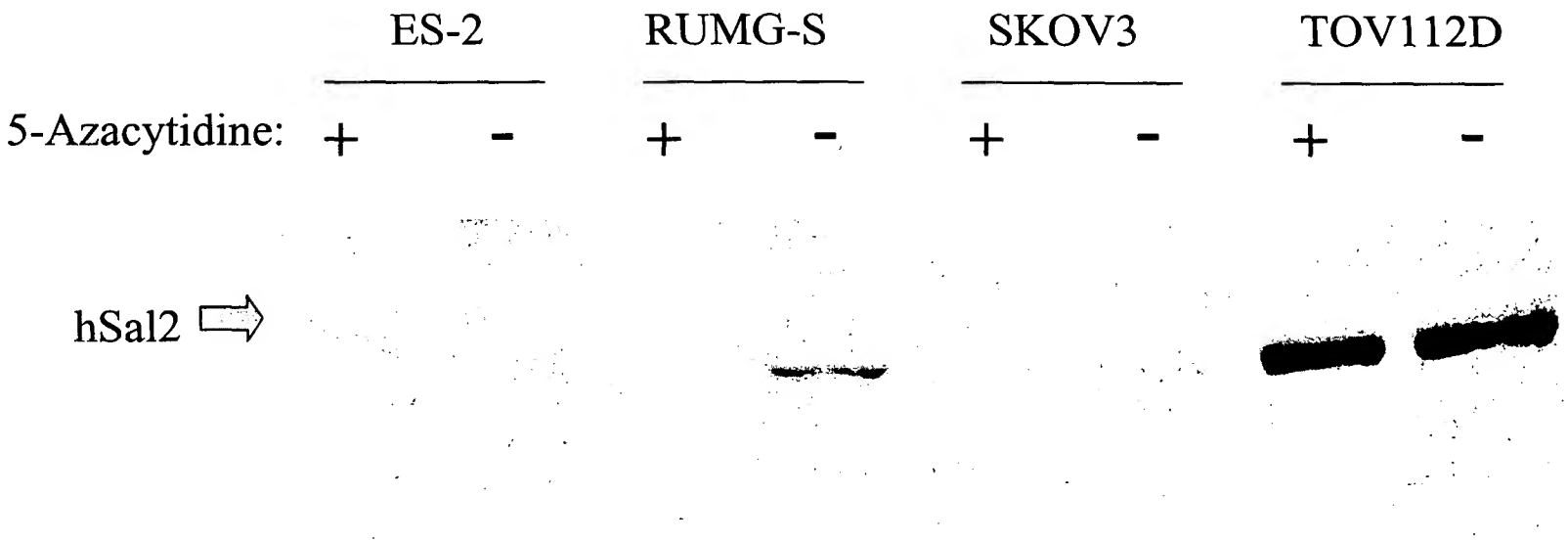


Figure 9. A loss of heterozygosity (LOH) variant of p150 is unable to induce p21 transcription. A. p150 LOH in the ovarian tumor of a 73S/C patient. Left: Schematic presentation of an amplicon of 318 bp covering the coding region of amino acid 73 of human p150 gene. Mbo II digestion of this amplicon generates 3 fragments (171, 94 and 53 bp) for the predominant 73S allele but 2 fragments (265 bp and 53 bp) for the 73C allele. Right: Matching DNA from normal tissues (N) and ovarian tumors (T) of 5 patients (numbered 1-5) were amplified to generate the 318 bp amplicon and digested with Mbo II and resolved on argrose gel. U: uncut control amplicaon. S: Mbo II digested 73S allele. C: Mbo II digested 73C allele. S/C: 73 S and 73C heterozygous alleles. **B.** 73C Variant has a diminished ability to induce p21 promoter. Left: Comparison of P21 promoter inducibility of p150 expression constructs of 73S allele (p150 73S) or 73C allele (p150 73C). Empty vector and p53 were used as controls. Right: Western blot shows similar expression levels of the transfected p150 (top band of the doublet).

Demethylation Increases hSal2 Expression in Some Ovarian Tumor Lines



Treatment by 5-azacytidine increased $P150^{hSal2}$ expression greatly in RUMG-S cells and significantly in SKOV3 cells. This treatment had no effect on hSal2 expression in ES-2 cells and TOV112D cells, which over expressed hSal2. Ovarian cell lines were treated with 2 uM 5-Azacytidine for 5 days. Each lane contains 50 ug of whole cell Extracts. The Western blot was probed with a polyclonal antibody against 3' hSal2.

Exhibit 6

Suppression of HPV-16 replication origin replication by hSal2 in C33A cells

HPV116 Ori	+	+	+	+
HPV-16 E1, E2	-	+	+	+
hSal2	-	-	+(1x)	+(2x)
% Replicated DNA	3.37	45.47	33.03	26.23
% Suppressed DNA	92.6	0	26.76	42.16

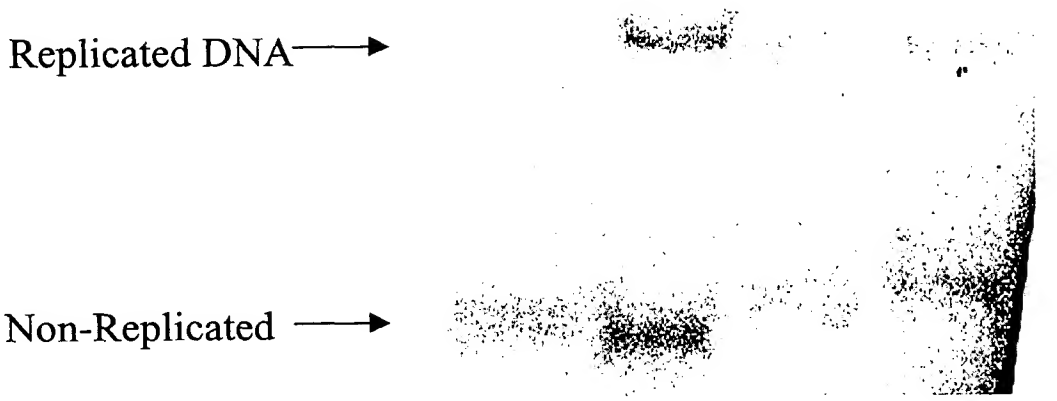


Exhibit 7